

CLAIMS

1. A purified thrombospondin fragment that has been extracted from a bodily fluid, said fragment being one within a molecular weight range selected from the group consisting of 80 to 110 kDa, 40 to 60 kDa, and 20 to 35 kDa, wherein the size in kDa is that determined by gel electrophoresis after disulfide bond reduction.

2. A purified thrombospondin fragment of Claim 1, wherein the bodily fluid is blood plasma.

3. A purified thrombospondin fragment of Claim 1, wherein the fragment's molecular weight is one within a molecular weight range selected from the group consisting 85 to 90 kDa fragment, 47 to 53 kDa, and 27 to 33 kDa.

4. A molecule identical in primary structure to the compound of Claim 2.

5. A fragment of Claim 4 further modified to have a modification selected from the group consisting of glycosylation, deglycosylation, β -hydroxylation, alkylation, reduction, denaturation, renaturation, calcium depletion, calcium supplementation, conjugation, and addition of groups or moieties to aid conjugation, stability, and/or immunogenicity.

6. A purified and/or synthetic thrombospondin fragment or portion thereof, said fragment selected from the group comprising one that starts between amino acyl residues I-165 and V-263, inclusive, and ends between amino acyl residues K-412 and I-530, inclusive;
one that starts between amino acyl residues I-165 and V-263, inclusive, and ends between amino acyl residues I-530 and R-733, inclusive;

and one that starts between amino acyl residues I-165 and V-263, inclusive, and ends between amino acyl residues R-792 and Y-982, inclusive, said portion being at least 4 amino acyl acids in length.

7. A purified and/or synthetic thrombospondin fragment or portion thereof, selected from the group comprising

one that starts between amino acyl residues N-230 and G-253 inclusive, and ends between amino acyl residues V-400 and S-428, inclusive;

one that starts between amino acyl residues N-230 and G-253, inclusive, and ends between amino acyl residues D-527 and S-551, inclusive;

and one that starts between amino acyl residues N-230 and G-253, inclusive, and ends between amino acyl residues G-787 and V-811, inclusive, said portion being at least 4 amino acyl acids in length.

8. A purified thrombospondin fragment, the molecular weight of said fragment not exceeding 100 kDa, said fragment comprising at least 4 contiguous amino acyl residues from the thrombospondin sequence, wherein the amino acid sequence of the fragment is limited to the group consisting of a fragment that is outside of a thrombospondin region defined in claim 6 and a fragment that is outside of a thrombospondin region defined in claim 7.

9. A purified and/or synthetic thrombospondin fragment, the molecular weight of said fragment not exceeding 110 kDa, said fragment being at least 4 contiguous amino acyl residues in length, and wherein the fragment comprises a domain or a part thereof within the protease-resistant core of thrombospondin, said domain being selected from the group consisting of a domain of inter-chain disulfide bonds, an oligomerization domain, a procollagen-like domain, a type 1 repeat, a type 2 repeat, and a type 3 repeat.

10. A purified and/or synthetic thrombospondin fragment, the molecular weight of said fragment not exceeding 110 kDa, said fragment being at least 4 contiguous amino acyl residues in length, and wherein the fragment comprises an amino acid sequence selected from the group consisting of TEENKE (SEQ ID NO: 1), CLQDSIRKVTEENKE (SEQ ID NO: 2), LQDSIRKVTEENKE (SEQ ID NO: 3), EGEARE (SEQ ID NO: 4), PQMNGKPCEGEARE (SEQ ID NO: 5), EDTDLD (SEQ ID NO: 6), YAGNGIICGEDTDLD (SEQ ID NO: 7), CNSPSPQMNGKPCEGEAR (SEQ ID NO: 8), RKVTEENKELANELRRP (SEQ ID NO: 9), CRKVTEENKELANELRRP (SEQ ID NO: 10), PQMNGKPCEGEAR (SEQ ID NO: 11), CEGEAR (SEQ ID NO: 12), RKVTEENKE (SEQ ID NO: 13), TERDDD (SEQ ID NO: 24), DFSGTFFINTERDDD (SEQ ID NO: 25), ERKDHHS (SEQ ID NO: 26), TRGTLLALERKDHHS (SEQ ID NO: 27), CTRGTLLALERKDHHS (SEQ ID NO: 28), DDKFQD (SEQ ID NO: 29), ANLIPPVPDDKFQD (SEQ ID NO: 30), CANLIPPVPDDKFQD (SEQ ID NO: 31), DCEKME (SEQ ID NO: 32), EDRAQLYIDCEKMEN (SEQ ID NO: 33), CGTNRIPESGGDNSVFD (SEQ ID NO: 34), NRIPESGGDNSVFD (SEQ ID NO: 35), GWKDFTAYRWRLSHRPKTG (SEQ ID NO: 36), CGWKDFTAYRWRLSHRPKTG (SEQ ID NO: 37), DDDDNDKIPDDRDNK (SEQ ID NO: 14), DDDDNDKIPDDRDNK[NH₂] (SEQ ID NO: 15), DDDDNDK (SEQ ID NO: 16), NLPNSGQEDYDKDG (SEQ ID NO: 17), CNLPNSGQEDYDKDG (SEQ ID NO: 18), EDYDKD (SEQ ID NO: 19), CPYNHNPQADTDNNGEGD (SEQ ID NO: 20), CRLVPNPQDKSDGD (SEQ ID NO: 21), DQKSDGD (SEQ ID NO: 22), CPYVPNANQADHDKDGKGDA (SEQ ID NO: 23), and a portion of such an amino acid sequence.

11. A purified and/or synthetic thrombospondin fragment, the molecular weight of said fragment not exceeding 110 kDa, said fragment being at least 4 contiguous amino acyl residues in length, and wherein the fragment comprises a portion of a collagen type V binding domain.

12. A purified and/or synthetic thrombospondin fragment, the molecular weight of said fragment not exceeding 110 kDa, said fragment being at least 4 contiguous amino acyl residues in length, and wherein the fragment comprises an epitope for binding the TSP Ab-4 antibody.

13. A purified and/or synthetic thrombospondin fragment, the molecular weight of said fragment not exceeding 110 kDa, said fragment being at least 4 contiguous amino acyl residues in length, and wherein the fragment does not comprise a fibrinogen-binding region selected from the group consisting of (1) a fibrinogen-binding domain within a 210-kDa fragment of TSP, where said 210-kDa fragment is composed of three 70-kDa fragments that contain the region of interchain disulfide bonds, the procollagen homology region, and the type 1 and type 2 repeats, (2) a fibrinogen-binding region in the amino-terminal domain of thrombospondin, (3) a fibrinogen-binding region in an 18-kDa amino-terminal heparin-binding domain of thrombospondin, and (4) a region corresponding to synthetic peptide N12/I encompassing amino acid residues 151-164 (I-151 to P-164) of the N-terminal domain of thrombospondin-1.

14. A fragment of Claims 6,7, 8, 9,10,11,12 or 13, said fragment being at least 6 contiguous amino acyl residues in length.

15. A fragment of Claims 6,7,8,9,10,11,12 or 13, wherein the molecular weight of the fragment does not exceed 55 kDa.

16. A fragment of Claims 5 6,7,8,9,10,11,12 or 13, wherein the molecular weight of the fragment does not exceed 35 kDa.

17. A fragment or portion thereof specified in Claims 6,7,8, 9,10,11,12 or 13, said fragment or portion comprising at least 13 contiguous amino acyl residues.

18. A fragment or portion thereof specified in Claims 6,7,8, 9,10,11,12 or 13, said fragment or portion being a synthetic peptide, said peptide being either glycosylated or nonglycosylated, and/or either β -hydroxylated or not β -hydroxylated.

19. A thrombospondin fragment or portion thereof specified in Claims 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 that comprises a detectable label, said label being either intrinsic or an added moiety.

20. A thrombospondin fragment or portion thereof specified in Claim 1, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13, wherein said fragment or portion comprises a detectable label that is selected from the group consisting of a radioactive label, a fluorescent label, a chemical label, a colorimetric label, an enzymatic label, a non-fluorescent label, a non-radioactive label, a biotin moiety, and an avidin moiety.

21. A method to detect and/or quantify a thrombospondin fragment of Claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13 or a fragment portion of Claim 6 or 7.

22. A method of claim 21, wherein the method distinguishes the thrombospondin fragment or fragment portion from thrombospondin.

23. A method of Claim 22 wherein the method comprises a step wherein the fragment or fragment portion is physically separated from the thrombospondin.

24. A method of Claim 23 wherein the physical separation is accomplished using a technique that is selected from the group consisting of gel electrophoresis, dialysis, chromatography, size chromatography, affinity chromatography, immunoaffinity chromatography, adsorption, immunoadsorption, isoelectric focusing, mass spectrometry, centrifugation, sedimentation, floatation, precipitation, immunoprecipitation, and gel filtration.

25. A method of Claim 22 that distinguishes the fragment or fragment portion based on one or more epitopes in the fragment or fragment portion that are not present in thrombospondin.

26. A method of Claim 22 that distinguishes the fragment or fragment portion based on one or more epitopes in thrombospondin that are not present in the fragments, said method comprising the steps of:

1) utilizing an epitope shared by thrombospondin and the thrombospondin fragment or thrombospondin portion as a target for a binding molecule, such as an antibody, to obtain a quantitation of a total, thrombospondin plus either the thrombospondin fragment or thrombospondin portion,

2) utilizing an epitope present in thrombospondin but not present in the fragment or thrombospondin portion to obtain a quantitation of thrombospondin only; and

3) utilizing the difference between the quantitations obtained in steps (1) and (2) as a quantitation of the amount of fragment or thrombospondin portion.

27. The method of Claim 26 wherein in Step 1 the epitope is contained within an amino acid sequence selected from the group consisting of a fragment of claim 6, a fragment

of claim 7, a fragment of claim 9, a fragment of claim 11, a fragment of claim 12, TEENKE (SEQ ID NO: 1), CLQDSIRKVTEENKE (SEQ ID NO: 2), LQDSIRKVTEENKE (SEQ ID NO: 3), EGEARE (SEQ ID NO: 4), PQMNGKPCEGEARE (SEQ ID NO: 5), EDTDLD (SEQ ID NO: 6), YAGNGHICGEDTDLD (SEQ ID NO: 7), CNSPSPQMNGKPCEGEAR (SEQ ID NO: 8), RKVTEENKELANELRRP (SEQ ID NO: 9), CRKVTEENKELANELRRP (SEQ ID NO: 10), PQMNGKPCEGEAR (SEQ ID NO: 11), CEGEAR (SEQ ID NO: 12), RKVTEENKE (SEQ ID NO: 13), DDDDNDKIPDDRDNC (SEQ ID NO: 14), DDDDNDKIPDDRDNC[NH₂] (SEQ ID NO: 15), DDDDNDK (SEQ ID NO: 16), NLPNSGQEDYDKDG (SEQ ID NO: 17), CNLPNSGQEDYDKDG (SEQ ID NO: 18), EDYDKD (SEQ ID NO: 19), CPYNHNPDAQADTDNNGEGD (SEQ ID NO: 20), CRLVPNPDQKSDGD (SEQ ID NO: 21), DQKSDGD (SEQ ID NO: 22), CPYVPNANQADHDKDGKGDA (SEQ ID NO: 23), and a portion, at least 3 amino acyl residues in length of such an amino acid sequence.

28. The method of Claim 26 wherein in Step 2 the epitope is contained within an amino acid sequence selected from the group consisting of a fragment of claim 8, TERDDD (SEQ ID NO: 24), DFSGTFFINTERDDD (SEQ ID NO: 25), ERKDHS (SEQ ID NO: 26), TRGTLLALERKDHS (SEQ ID NO: 27), (C)TRGTLLALERKDHS (SEQ ID NO: 28), DDKFQD (SEQ ID NO: 29), ANLIPPVPDDKFQD (SEQ ID NO: 30), (C)ANLIPPVPDDKFQD (SEQ ID NO: 31), DCEKME (SEQ ID NO: 32), EDRAQLYIDCEKMEN (SEQ ID NO: 33), CGTNRIPESGGDNSVFD (SEQ ID NO: 34), NRIPESGGDNSVFD (SEQ ID NO: 35), GWKDFTAYRWRLSHRPKTG (SEQ ID NO: 36), CGWKDFTAYRWRLSHRPKTG (SEQ ID NO: 37), DDDDNDKIPDDRDNC (SEQ ID NO: 14), DDDDNDKIPDDRDNC[NH₂] (SEQ ID NO: 15), DDDDNDK (SEQ ID NO: 16), NLPNSGQEDYDKDG (SEQ ID NO: 17), CNLPNSGQEDYDKDG (SEQ ID NO: 18), EDYDKD (SEQ ID NO: 19), CPYNHNPDAQADTDNNGEGD (SEQ ID NO: 20), YAGNGHICGEDTDLD (SEQ ID NO: 7), EDTDLD (SEQ ID NO: 6), CNSPSPQMNGKPCEGEAR (SEQ ID NO: 8), PQMNGKPCEGEARE (SEQ ID NO: 5), EGEARE (SEQ ID NO: 4), PQMNGKPCEGEAR (SEQ ID NO: 11), CEGEAR (SEQ ID NO: 12), CRLVPNPDQKSDGD (SEQ ID NO: 21), DQKSDGD (SEQ ID NO: 22), CPYVPNANQADHDKDGKGDA (SEQ ID NO: 23), and a portion, at least 3 amino acyl residues in length of such an amino acid sequence, provided that said peptide with a designated SEQ ID NO. does not comprise an epitope used in step 1

29. A method of Claim 21 wherein the method is applied to a sample of material taken or gathered from an organism.

30. A method of Claim 21 wherein the organism is a human.

31. A method of Claim 21 wherein the organism is a nonhuman.

32. A method of Claim 21 wherein the method is applied to a sample of human plasma.

33. A method of Claim 21 wherein the method is performed in order to detect the presence of, or monitors the course of, a disease or condition.

34. A method of Claim 33 wherein the disease or condition is selected from the group consisting of a cancer, renal failure, renal disease, atopic dermatitis, vasculitis, acute vasculitis, renal allograft, asthma, diabetes mellitus, myocardial infarction, liver disease, splenectomy, dermatomyositis, polyarteritis nodosa, systemic lupus erythematosus, lupus erythematosus, Kawasaki syndrome, non-specific vasculitis, juvenile rheumatoid arthritis, rheumatoid arthritis, vasculitis syndrome, Henoch-Schönlein purpura, thrombocytopenic purpura, purpura, an inflammatory condition, a condition associated with clotting, a condition associated with platelet activation, a condition associated with intravascular platelet activation, a condition associated with consumption of platelets, heparin-induced thrombocytopenia, disseminated intravascular coagulation, intravascular coagulation, extravascular coagulation, a condition associated with endothelial activation, a condition associated with production and/or release of thrombospondin and/or a thrombospondin fragment, urticaria, hives, angioedema, a drug reaction, an antibiotic reaction, an aspartame reaction, atopic dermatitis, eczema, hypersensitivity, scleroderma, conditions associated with plugging of vessels, a condition associated with a cryofibrinogen, a condition associated with a cryoglobulin, and a condition associated with an anti-cardiolipin antibody.

35. A method of Claim 33 wherein the disease is a cancer.

36. A method of Claim 35 wherein the cancer is selected from the group consisting of an adenoma, adenocarcinoma, carcinoma, lymphoma, leukemia, solid cancer, liquid cancer, metastatic cancer, pre-metastatic cancer, non-metastatic cancer, a cancer with vascular invasion, internal cancer, skin cancer, cancer of the respiratory system, cancer of the circulatory system, cancer of the musculoskeletal system, cancer of a muscle, cancer of a bone, cancer of a joint, cancer of a tendon or ligament, cancer of the digestive system, cancer of the liver or biliary system, cancer of the pancreas, cancer of the head, cancer of the neck, cancer of the endocrine system, cancer of the reproductive system, cancer of the male

reproductive system, cancer of the female reproductive system, cancer of the genitourinary system, cancer of a kidney, cancer of the urinary tract, cancer of a sensory system, cancer of the nervous system, cancer of a lymphoid organ, blood cancer, cancer of a gland, cancer of a mammary gland, cancer of a prostate gland, cancer of an endometrial tissue, cancer of a mesodermal tissue, cancer of an ectodermal tissue, cancer of an endodermal tissue, a teratoma, a poorly-differentiated cancer, a well-differentiated cancer, and a moderately differentiated cancer.

37. A method of Claim 21 wherein the method is performed to measure the degree of platelet activation.

38. A method of Claim 21 wherein the method is performed to measure the degree of secretion of thrombospondin and/or a thrombospondin fragment from a tissue.

39. A method of claim 38 wherein the tissue is selected from the group consisting of a cancer, a neoplasm, an activated endothelium, and a stroma.

40. A method of Claim 21 wherein the method is performed on plasma that was obtained by a method that prevents or reduces platelet activation and/or protease activity during sample collection and/or storage.

41. A method of Claim 40 wherein the method does not comprise the use of a tourniquet and/or stasis.

42. A method of Claim 40 wherein the method comprises a step selected from the group consisting of: (1) use of a large-bore needle, (2) discarding of the initial portion of the collected blood, (3) use of a coated needle, (4) use of a coated tubing, (5) storage of sample between -1°C and 5°C, and (6) separation of plasma from cellular components of blood within 30 minutes of sample collection.

43. A method of Claim 40 wherein the method comprises the use of an agent selected from the group consisting of a platelet inhibitor, a protease inhibitor, a serine protease inhibitor, an enzyme inhibitor, an inhibitor of an enzyme that is divalent cation dependent, a heparin, a heparin fragment, a low-molecular weight heparin, a heparan, a heparan sulfate, an anticoagulant, a COX inhibitor, an inhibitor of a cell-adhesion molecule, an inhibitor of a surface receptor, a glycoprotein inhibitor, an inhibitor of a glycoprotein IIb/IIIa receptor, a thrombin inhibitor, an inhibitor of degranulation, a chelator, a citrate compound, theophylline, adenosine, and dipyridamole.

44. A method of Claim 21 or claim 33, said method further comprising an method not based on a thrombospondin fragment or portion thereof.

45. A method of Claim 44 wherein the test not based on a thrombospondin fragment or portion thereof is selected from the group consisting of an imaging test, a radiographic test, a nuclear medicine test, a magnetic resonance imaging test, a blood test, a biopsy, a histologic test, a cytologic test, an immunohistologic test, a genetic test, a guaiac test, a test for fecal occult blood, a test for fecal blood, a test for fecal DNA, a test for a fecal cancer marker, a cancer test not based on a thrombospondin fragment or portion thereof, a receptor test, an estrogen receptor test, a disease test not based on a thrombospondin fragment or portion thereof, an endoscopy, an upper gastrointestinal endoscopy, a lower gastrointestinal endoscopy, a colonoscopy, a sigmoidoscopy, a gastroscopy, a laparoscopy, a laparotomy, a lymph node biopsy, a surgery, and a bronchoscopy.

46. A method of Claim 45 wherein the blood test is selected from the group consisting of a cancer antigen test, a cancer gene test, a cancer DNA test, a cancer mRNA test, a cancer RNA test, a cancer protein test, a cancer glycoprotein test, a cancer carbohydrate test, a cancer lipid test, a prostate specific antigen test, a test of carcinoembryonic antigen, a test of cancer antigen CA-125, a test of alpha-fetoprotein, a test of CA15-3, a test of CA19-9, a test of malignin, a test of anti-malignin antibody, a test of anti-secretory factor, a cancer antigen that contains a carbohydrate epitope, a cancer antigen that contains a protein or polypeptide epitope, a cancer antigen that contains a lipid epitope, a cancer antigen that contains a mixed epitope, CA 27.29, and episialin.

47. A method of Claim 36, said method further comprising an method not based on a thrombospondin fragment or portion thereof.

48. A method of Claim 47, wherein the test not based on a thrombospondin fragment or portion thereof is selected from the group consisting of an imaging test, a radiographic test, a nuclear medicine test, a magnetic resonance imaging test, a blood test, a biopsy, a histologic test, a cytologic test, an immunohistologic test, a genetic test, a guaiac test, a test for fetal occult blood, a test for fecal blood, a test for fecal DNA, a test for a fecal cancer marker, a cancer test not based on a thrombospondin fragment or portion thereof, a receptor test, an estrogen receptor test, a disease test not based on a thrombospondin fragment or portion thereof, an endoscopy, an upper gastrointestinal endoscopy, a lower gastrointestinal endoscopy, a colonoscopy, a sigmoidoscopy, a gastroscopy, a laparoscopy, a laparotomy, a lymph node biopsy, a surgery, and a bronchoscopy.

49. A method of Claim 48 wherein the blood test is selected from the group consisting of a cancer antigen test, a cancer gene test, a cancer DNA test, a cancer mRNA

test, a cancer RNA test, a cancer protein test, a cancer glycoprotein test, a cancer carbohydrate test, a cancer lipid test, a prostate specific antigen test, a test of carcinoembryonic antigen, a test of cancer antigen CA-125, a test of alpha-fetoprotein, a test of CA15-3, a test of CA19-9, a test of malignin, a test of anti-malignin antibody, a test of anti-secretory factor, a cancer antigen that contains a carbohydrate epitope, a cancer antigen that contains a protein or polypeptide epitope, a cancer antigen that contains a lipid epitope, a cancer antigen that contains a mixed epitope, CA 27.29, and episialin.

50. A method of Claim 21 or Claim 33 wherein the thrombospondin fragment or portion thereof comprise a detectable label.

51. A method of Claim 50 wherein the thrombospondin fragment or portion thereof being detected is a target and/or indicator fragment and wherein a known or unknown amount of an unlabeled or differently labeled fragment is also subjected to the method, said unlabeled or differently labeled fragment being a thrombospondin fragment or portion thereof.

52. A method of Claim 51 wherein the amount of the unlabeled or differently labeled fragment is known.

53. A method of Claim 51 wherein the amount of the unlabeled or differently labeled fragment is unknown.

54. A method of producing antibodies against a thrombospondin fragment of Claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13 or a fragment portion of Claim 6 or Claim 7 said method comprising administering said fragment, fragment portion or immunogenic portion thereof to an organism capable of producing antibodies.

55. A method of Claim 54 wherein polyclonal antibodies are produced.

56. A method of Claim 54 wherein monoclonal antibodies are produced.

57. An antibody produced by the method of Claim 54.

58. A cell line producing the monoclonal antibodies of claim 56.

59. The cell line of claim 58, wherein said cell line is selected from the group consisting of a hybridoma, a transfected cell line, and an infected cell.

60. A method of producing a peptide or non-peptide binding agent against a thrombospondin fragment of Claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13, a fragment portion of Claim 6 or Claim 7, or epitope therein, said method comprising the steps of

1) a generating step (random, semi-random, directed, combinatorial, and/or other) to generate large numbers (>100) of diverse peptides and/or non-peptides;

2) a selection step to identify within this large number those peptides and/or non-peptides that bind to the thrombospondin fragment, fragment portion, and/or an epitope therein; and
3) optionally an improvement step for improving the peptide or non-peptide binding agent to achieve better affinity and/or specificity.

61. A method of Claim 60 wherein the binding agent is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a single-chain antibody, a non-antibody, a protein, a product of phage display, an aptamer, a DNA, an RNA, a modified DNA, a modified RNA, a carbohydrate, a glycosaminoglycan, a heparin, a glycoprotein, a proteoglycan, and combinations and derivatives thereof.

62. A method of Claim 60 wherein the optional step for improving the binding agent is selected from the group consisting of molecular evolution, mutation of crucial residues, making dimeric, trimeric or multimeric molecules, and incorporation of sequences from animals or humans exposed to or expressing antibodies against the fragment, portion, or epitope therein.

63. A method of Claim 60 wherein the initial set of diverse molecules is enriched by using sequences from animals or humans exposed to or expressing antibodies against the target.

64. A cell line capable of producing a binding agent produced by the method of claim 60.

65. The cell line of claim 64, wherein said cell line is selected from the group consisting of a hybridoma, a transfected cell line, and an infected cell.

66. A kit for the determination of the presence of, and/or the amount of, and/or the concentration of, a thrombospondin fragment in a material taken or gathered from an organism, said kit comprising a thrombospondin fragment of Claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13 or a fragment portion of Claim 6 or Claim 7.

67. A kit for the determination of the presence of, and/or the amount of, and/or the concentration of, one or more thrombospondin fragments in a material taken or gathered from an organism, said kit comprising a binding agent capable of binding said one or more of said fragments.

68. A kit of Claim 67 wherein said binding agent is a binding agent produced by the method of Claim 54 or Claim 60.

69. A kit of Claim 68 wherein said binding agent is a binding agent produced by the method of Claim 53 and/or the method of claim 54 and/or the method of 60 that binds to a thrombospondin fragment or epitope of claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and/or 13.

70. A kit of Claim 67 wherein said binding agent comprises a protein.

71. A kit of Claim 70 wherein said protein comprises a multi-chain antibody.

72. A kit of Claim 71 wherein said multi-chain antibody is selected from the group consisting of a monoclonal antibody and a polyclonal antibody.

73. A kit of Claim 70 wherein said protein comprises a fragment of a multi-chain antibody.

74. A kit of Claim 70 wherein said protein comprises a single-chain antibody.

75. A kit of Claim 67 wherein said binding agent is derived from a phage display library.

76. A kit of Claim 70 wherein said protein is a non-antibody, said non-antibody being a protein that is neither a multi-chain antibody nor a single-chain antibody.

77. A kit of Claim 76 wherein said non-antibody is selected from the group consisting of a thrombospondin receptor, a thrombospondin binding protein, a thrombospondin receptor and/or binding protein that binds within a protease-resistant core region, a thrombospondin receptor and/or binding protein that binds a TSP fragment present in the plasma of a cancer patient, a CSVTCG receptor, a CSVTCG binding molecule, an anti-secretory factor, an angiocidin, a 26S proteasome non-ATPase regulatory subunit 4, a CD36, a fragment thereof, a fragment thereof that binds to the respective target, and combinations, chimeras, and recombinant versions of said receptors and fragments.

78. A kit for the determination of the presence of, and/or the amount of, and/or the concentration of, one or more thrombospondin fragments in a material taken or gathered from an organism, said kit comprising a binding agent that will react with thrombospondin but not with the fragment or fragments of interest.

79. A kit of Claim 78 wherein said binding agent is a binding agent produced by the method of Claim 54 or Claim 60.

80. An method method comprising the following steps: (1) the method of Claim 52; (2) the method of Claim 53; and (3) a determination of the amount of the unlabeled or differently labeled fragment of claim 53 (the unknown) through comparison to the results obtained from the unlabeled or differently labeled fragment of claim 52 (the calibrator).

81. A kit of Claim 78 wherein said binding agent comprises a protein.

82. A kit of Claim 81 wherein said protein comprises a multi-chain antibody.
83. A kit of Claim 82 wherein said multi-chain antibody is selected from the group consisting of a monoclonal antibody and a polyclonal antibody.
84. A kit of Claim 81 wherein said protein comprises a fragment of a multi-chain antibody.
85. A kit of Claim 81 wherein said protein comprises a single-chain antibody.
86. A kit of Claim 81 wherein said protein is derived from a phage display library.
87. A kit of Claim 81 wherein the protein is a non-antibody, a non-antibody being a protein that is neither an antibody nor a single-chain antibody.
88. A kit of Claim 87 wherein said non-antibody is selected from the group consisting of an integrin, an RGD receptor, an RFYVVMWK receptor, an RFYVVM receptor, an FYVVMWK receptor, an IRVVM receptor, a CSVTCG receptor, a CSVTCG binding molecule, CD36, anti-secretory factor, angiocidin, 26S proteasome non-ATPase regulatory subunit 4, a fragment thereof that binds to the respective target, and combinations, chimeras, and recombinant versions of said receptors, integrins, and fragments.
89. A kit of Claim 67 wherein said binding agent comprises a non-protein.
90. A method of claims 21, 33, 54, or 60, wherein said thrombospondin fragment or portion thereof has been derivatized.
91. A method of claim 90, wherein said derivatized fragment or portion thereof comprises a moiety selected from the group consisting of a label, a carrier, an albumin carrier, a keyhole limpet hemocyanin carrier, an epitope tag, an epitope, and an adjuvant.
92. A method of claim 90, wherein the derivatization is selected from the group consisting of addition of a detectable label, incorporation of a detectable label, conjugation to another molecule, synthesis of the fragment as part of a chimeric protein, linkage to a carrier molecule or particle, linkage to a carrier, linkage to a bead, linkage to a solid matrix, linkage to keyhole limpet hemocyanin, linkage to an albumin, linkage to an ovalbumin, linkage to a cross-linking agent, linkage to an epitope tag, and linkage to an epitope.
93. A kit for the determination of the presence of, and/or the amount of, thrombospondin fragments in a material taken or gathered from an organism, said kit comprising an antibody that will react thrombospondin fragments of interest but not with thrombospondin.

94. A purified and/or synthetic thrombospondin fragment of Claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13 or a fragment portion of Claim 6 or Claim 7, said fragment or portion thereof being derivatized.

95. A purified and/or synthetic fragment or portion thereof of Claim 94, wherein the derivatization is selected from the group consisting of addition of a detectable label, incorporation of a detectable label, conjugation to another molecule, synthesis of the fragment as part of a chimeric protein, linkage to a carrier molecule or particle, linkage to a carrier, linkage to a bead, linkage to a solid matrix, linkage to keyhole limpet hemocyanin, linkage to an albumin, linkage to an ovalbumin, linkage to a cross-linking agent, linkage to an epitope tag, and linkage to an epitope.

96. The fragment of claim 92 and/or Claim 95, wherein said label selected from the group consisting of a radioactive label, a fluorescent label, a chemical label, a colorimetric label, an enzymatic label, a non-fluorescent label, a non-radioactive label, a biotin moiety, and an avidin moiety.

97. The fragment of Claim 92 and/or Claim 95, wherein said carrier is a moiety selected from the group consisting of a bead, a microsphere, a coded microsphere, a solid matrix, keyhole limpet hemocyanin, an albumin, an ovalbumin, linkage to a cross-linking agent, an epitope tag, and an epitope.

98. A method of Claim 21 said method comprising the step of binding a binding agent to the thrombospondin fragment or portion thereof.

99. A method of Claim 98 wherein said binding agent is a binding produced by the method of Claim 54 or 60.

100. A method of Claim 98 wherein said binding agent is a binding agent produced by the method of Claim 54 and/or Claim 60.

101. A method of Claim 100 wherein said binding agent comprises a protein and/or a polypeptide.

102. A method of Claim 101 wherein said protein comprises an antibody.

103. A method of Claim 102 wherein said antibody is selected from the group consisting of a monoclonal antibody and a polyclonal antibody.

104. A method of Claim 101 wherein said protein and/or polypeptide comprises an antibody fragment.

105. A method of Claim 101 wherein said protein comprises a single chain antibody.

106. A method of Claim 101 wherein said protein and/or polypeptide is derived from a phage display library.

107. A method of Claim 101 wherein said protein is a non-antibody, said non-antibody being a protein that is neither a multi-chain antibody nor a single-chain antibody.

108. A method of Claim 107 wherein said non-antibody is selected from the group consisting of a thrombospondin receptor, a thrombospondin binding molecule, a thrombospondin receptor and/or binding molecule that binds within a protease-resistant core region, a thrombospondin receptor and/or binding molecule that binds a TSP fragment present in the plasma of a cancer patient, a CSVTCG receptor, a CSVTCG binding molecule, an anti-secretory factor, a CD36, a fragment thereof, a fragment thereof that binds to the respective target, and combinations, chimeras, and recombinant versions of said receptors and fragments.

109. A method of Claim 98 wherein said binding agent is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a single-chain antibody, a non-antibody, a protein, a product of phage display, an anti-secretory factor, an aptamer, a DNA, an RNA, a modified DNA, a modified RNA, a carbohydrate, a glycosaminoglycan, a heparin, a glycoprotein, a proteoglycan, and combinations and derivatives thereof.

110. A method to detect the presence and/or clinical course of a neoplastic disease in an individual, wherein the method comprises the steps of:

(1) measuring the individual's plasma level of a thrombospondin fragment or fragments;

(2) utilizing the result of step (1) in a diagnosis as to whether the individual has a neoplastic disease; said fragment or fragments being a fragment of claims 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12 and/or 13, and/or comprising an epitope therein.

111. A method of Claim 110 where the individual referred to therein is a first individual and wherein the method further comprises the steps of:

(3) measuring a second individual's plasma level of the thrombospondin fragment, said second individual considered to not have neoplastic disease;

(4) utilizing the result of step (3) in the diagnosis of whether the first individual has a neoplastic disease.

112. A method of Claim 111, wherein the greater the extent to which the first individual's plasma thrombospondin fragment level exceeds the plasma thrombospondin level of the second individual, the more likely that the diagnosis will be that the first

individual has a neoplastic disease and/or a neoplastic disease more advanced than that of the second person.

113. A method of Claim 110 further comprising the steps of assaying the individual's plasma level of a thrombospondin fragment more than once, and utilizing the change in plasma level from an older to a more recent value to indicate appearance or progression or improvement wherein said appearance or progression is indicated by an increase in the plasma level and said improvement is indicated by a decrease in said plasma level.

114. A method of Claim 113 wherein the plasma level of a thrombospondin fragment is assayed repeatedly.

115. A method of claim 114 wherein the repeated assays are performed at regular intervals, said intervals ranging from two weeks to 10 years.

116. A method of Claim 109, 110, 111, 112, 113, 114 or 115, wherein the neoplastic disease is selected from the group consisting of an adenoma, adenocarcinoma, carcinoma, lymphoma, leukemia, and sarcoma.

117. A method of Claim 109, 110, 111, 112, 113, 114 or 115, wherein the neoplastic disease is an internal cancer.

118. A method of Claim 109, 110, 111, 112, 113, 114 or 115, wherein the neoplastic disease is selected from the group consisting of a cancer of the respiratory system, a cancer of the circulatory system, a cancer of the musculoskeletal system, a cancer of a muscle, a cancer of a bone, a cancer of a joint, a cancer of a tendon or ligament, a cancer of the digestive system, a cancer of the liver or biliary system, a cancer of the pancreas, a cancer of the head, a cancer of the neck, a cancer of the endocrine system, a cancer of the reproductive system, a cancer of the male reproductive system, a cancer of the female reproductive system, a cancer of the genitourinary system, a cancer of a kidney, a cancer of the urinary tract, a skin cancer, a cancer of other sensory organs, a cancer of the nervous system, a cancer of a lymphoid organ, a blood cancer, a cancer of a gland, a cancer of a mammary gland, a cancer of a prostate gland, a cancer of endometrial tissue, a cancer of mesodermal tissue, a cancer of ectodermal tissue, cancer of an endodermal tissue, a teratoma, a poorly-differentiated cancer, a well-differentiated cancer, and a moderately differentiated cancer.

119. A method of Claim 109, 110, 111, 112, 113, 114 or 115, wherein the neoplastic disease is selected from the group consisting of a cancer of solid tissue, a cancer of the blood or the lymphatic system, a solid cancer, a liquid cancer, a non-metastatic cancer, a premetastatic cancer, a metastatic cancer, a cancer with vascular invasion, a skin cancer, a

poorly differentiated cancer, a well-differentiated cancer and a moderately differentiated cancer.

120. A method of Claim 109, 110, 111, 112, 113, 114 or 115, wherein the measurement of a plasma thrombospondin fragment level comprises the use of a binding agent, said binding agent capable of binding said fragment.

121. A method of Claim 120 wherein said binding agent is a binding agent produced by the method of Claim 54 or 60.

122. A method of Claim 121 wherein said binding agent is a binding agent produced by a method of Claim 54 or Claim 60 that binds to a thrombospondin fragment or portion of claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and/or 13, or an epitope therein.

123. A method of Claim 110 wherein said binding agent comprises a protein and/or a polypeptide.

124. A method of Claim 123 wherein said protein comprises an antibody.

125. A method of Claim 124 wherein said antibody is selected from the group consisting of a monoclonal antibody and a polyclonal antibody.

126. A method of Claim 123 wherein said protein comprises an antibody fragment.

127. A method of Claim 123 wherein said protein comprises a single chain antibody.

128. A method of Claim 120 wherein said binding agent comprises a non protein.

129. A method of Claim 123 wherein said protein and/or polypeptide is derived from a phage display library.

130. A method of Claim 123 wherein said protein is a non-antibody, said non-antibody being a protein that is neither a multi-chain antibody nor a single-chain antibody.

131. A method of Claim 130 wherein said non-antibody is selected from the group consisting of a thrombospondin receptor, a thrombospondin binding protein, a thrombospondin receptor and/or binding protein that binds within a protease-resistant core region, a thrombospondin receptor and/or binding protein that binds a TSP fragment present in the plasma of a cancer patient, a CSVTCG receptor, a CSVTCG binding molecule, an anti-secretory factor, an angiocidin, a 26S proteasome non-ATPase regulatory subunit 4, a CD36, a fragment thereof, a fragment thereof that binds to the respective target, and combinations, chimeras, and recombinant versions of said receptors and fragments.

132. A method of Claim 110 wherein said binding agent is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a single-chain antibody, a non-antibody, a protein, a product of phage display, an anti-secretory factor, an aptamer, a DNA,

an RNA, a modified DNA, a modified RNA, a carbohydrate, a glycosaminoglycan, a heparin, a glycoprotein, a proteoglycan, and combinations and derivatives thereof.

133. A method of Claim 121 wherein the thrombospondin fragment is separated from thrombospondin before said fragment is bound to the binding agent.

134. A method of Claim 110 wherein said method comprises the use of a binding agent, comprising a binding agent capable of binding thrombospondin but not the thrombospondin fragment.

135. A method of Claim 134 wherein said binding agent is a binding agent produced by the method of Claim 54 or 60.

136. A method of Claim 134 wherein said binding agent is a binding agent produced by the method of Claim 54 or 60 that binds to a thrombospondin fragment or portion of Claim 6, Claim 7, Claim 9, Claim 11, and/or Claim 12 or an epitope therein.

137. A method of Claim 134 wherein said binding agent comprises a protein and/or polypeptide.

138. A method of Claim 137 wherein said protein comprises an antibody.

139. A method of Claim 138 wherein said antibody is selected from the group consisting of a monoclonal antibody and a polyclonal antibody.

140. A method of Claim 137 wherein said protein comprises an antibody fragment.

141. A method of Claim 137 wherein said protein and/or polypeptide is derived from a phage display library.

142. A method of Claim 137 wherein said protein is a non-antibody, said non-antibody being a protein that is neither a multi-chain antibody nor a single-chain antibody.

143. A method of Claim 142 wherein said non-antibody is selected from the group consisting of an integrin, an RGD receptor, an RFYVVMWK receptor, an RFYVVM receptor, an FYVVMWK receptor, an IRVVM receptor, a CSVTCG receptor, a CSVTCG binding molecule, CD36, anti-secretory factor, angiocidin, 26S proteasome non-ATPase regulatory subunit 4, a fragment thereof that binds to the respective target, and combinations, chimeras, and recombinant versions of said receptors, integrins, and fragments.

144. A method of Claim 134 wherein said binding agent selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a single-chain antibody, a non-antibody, a protein, a product of phage display, an anti-secretory factor, an aptamer, a DNA, an RNA, a modified DNA, a modified RNA, a carbohydrate, a glycosaminoglycan, a heparin, a glycoprotein, a proteoglycan, and combinations and derivatives thereof.

145. A method of producing antibodies against a thrombospondin fragment, said fragment at least 6 amino acyl residues in length, said method comprising administering said fragment to an organism capable of producing antibodies.

146. A monoclonal antibody produced by the method of Claim 145.

147. A cell line producing a monoclonal antibody of claim 146.

148. A polyclonal antibody preparation produced by the method of Claim 145.

149. A method of producing a binding agent against a thrombospondin fragment, said fragment at least 6 amino acyl residues in length, said method comprising binding a phage to said thrombospondin fragment.

150. A fragment or portion thereof of Claims 6, 7, 8, 9, 10, 11, 12, or 13 further modified to have a modification selected from the group consisting of glycosylation, β -hydroxylation, and addition of groups or moieties to aid conjugation and/or stability.

151. A fragment or portion thereof of claim 5 or 150, wherein said groups or moieties are selected from the group consisting of addition of a cysteine residue and addition of a terminal amino group.

152. A purified thrombospondin fragment, said fragment comprising at least 6 contiguous amino acyl residues from the thrombospondin sequence, wherein the amino acid sequence of the fragment is limited to one that is outside of a thrombospondin region defined in claim 6, and/or claim 7.

153. A method of Claim 54, 60, 145, and/or 149 wherein said fragment, fragment portion, or immunogenic portion is derivatized.

154. A purified and/or synthetic thrombospondin fragment of Claim 10, wherein the fragment comprises an amino acid sequence selected from the group consisting of TEENKE (SEQ ID NO: 1), CLQDSIRKVTEENKE (SEQ ID NO: 2), LQDSIRKVTEENKE (SEQ ID NO: 3), EGEARE (SEQ ID NO: 4), PQMNGKPCEGEARE (SEQ ID NO: 5), EDTDLD (SEQ ID NO: 6), YAGNGHICGEDTDLD (SEQ ID NO: 7), CNSPSPQMNGKPCEGEAR (SEQ ID NO: 8), RKVTEENKELANELRRP (SEQ ID NO: 9), CRKVTEENKELANELRRP (SEQ ID NO: 10), PQMNGKPCEGEAR (SEQ ID NO: 11), CEGEAR (SEQ ID NO: 12), RKVTEENKE (SEQ ID NO: 13), and a portion at least 3 amino acyl residues in length (preferably at least 4 amino acyl residues in length, more preferably at least 6 amino acyl residues) of such an amino acid sequence.

155. A purified and/or synthetic thrombospondin fragment of Claim 10, wherein the fragment comprises an amino acid sequence selected from the group consisting of TERDDD

(SEQ ID NO: 24), DFSGTFFINTERDDD (SEQ ID NO: 25), ERKDHS (SEQ ID NO: 26), TRGTLLALERKDHS (SEQ ID NO: 27), CTRGTLLALERKDHS (SEQ ID NO: 28), DDKFQD (SEQ ID NO: 29), ANLIPPVPDDKFQD (SEQ ID NO: 30), CANLIPPVPDDKFQD (SEQ ID NO: 31), DCEKME (SEQ ID NO: 32), EDRAQLYIDCEKMEN (SEQ ID NO: 33), CGTNRIPESGGDNSVFD (SEQ ID NO: 34), NRIPESGGDNSVFD (SEQ ID NO: 35), GWKDFTAYRWRLSHRPKTG (SEQ ID NO: 36), CGWKDFTAYRWRLSHRPKTG (SEQ ID NO: 37), and a portion at least 3 amino acyl residues in length (preferably at least 4 amino acyl residues in length, more preferably at least 6 amino acyl residues) of such an amino acid sequence.

156. A purified and/or synthetic thrombospondin fragment of Claim 10, wherein the fragment comprises an amino acid sequence selected from the group consisting of DDDDNDKIPDDRDNC (SEQ ID NO: 14), DDDDNDKIPDDRDNC[NH₂] (SEQ ID NO: 15), DDDDNDK (SEQ ID NO: 16), NLPNSGQEDYDKDG (SEQ ID NO: 17), CNLPNSGQEDYDKDG (SEQ ID NO: 18), EDYDKD (SEQ ID NO: 19), CPYNHNPQADTDNNGEGD (SEQ ID NO: 20), CRLVPNPQKDSGD (SEQ ID NO: 21), DQKDSGD (SEQ ID NO: 22), CPYVPNANQADHDKDGGKGA (SEQ ID NO: 23) and a portion at least 3 amino acyl residues in length (preferably at least 4 amino acyl residues in length, more preferably at least 6 amino acyl residues) of such an amino acid sequence.

157. A kit of claim 89 wherein the non-protein is an aptamer.

158. A purified and/or synthetic thrombospondin fragment or portion thereof, said fragment selected from the group comprising one that starts between amino acyl residues I-165 and V-263, inclusive, and ends between amino acyl residues K-412 and I-530, inclusive; one that starts between amino acyl residues I-165 and V-263, inclusive, and ends between amino acyl residues I-530 and R-733, inclusive;

and one that starts between amino acyl residues I-165 and V-263, inclusive, and ends between amino acyl residues R-733 and Y-982, inclusive, said portion being at least 6 amino acyl acids in length.

159. A purified thrombospondin fragment, said fragment comprising at least 6 contiguous amino acyl residues from the thrombospondin sequence, wherein the amino acid sequence of the fragment is limited to one that is outside of a thrombospondin region defined in claim 6, claim 7, and/or claim 158.

160. The method of Claim 26 wherein in Step 1 the epitope comprises an amino acid sequence selected from the group consisting of a fragment of claim 6, a fragment of claim 7, a fragment of claim 9, a fragment of claim 11, a fragment of claim 12, TEENKE (SEQ ID NO: 1), CLQDSIRKVTEENKE (SEQ ID NO: 2), LQDSIRKVTEENKE (SEQ ID NO: 3), EGEARE (SEQ ID NO: 4), PQMNGKPCEGEARE (SEQ ID NO: 5), EDTDLD (SEQ ID NO: 6), YAGNGIICGEDTDLD (SEQ ID NO: 7), CNSPSPQMNGKPCEGEAR (SEQ ID NO: 8), RKVTEENKELANELRRP (SEQ ID NO: 9), CRKVTEENKELANELRRP (SEQ ID NO: 10), PQMNGKPCEGEAR (SEQ ID NO: 11), CEGEAR (SEQ ID NO: 12), RKVTEENKE (SEQ ID NO: 13), DDDDNDKIPDDRDNC (SEQ ID NO: 14), DDDDNDKIPDDRDNC[NH₂] (SEQ ID NO: 15), DDDDNDK (SEQ ID NO: 16), NLPNSGQEDYDKDG (SEQ ID NO: 17), CNLPNSGQEDYDKDG (SEQ ID NO: 18), EDYDKD (SEQ ID NO: 19), CPYNHNPQADTDNNGEGD (SEQ ID NO: 20), CRLVPNPQKDSGD (SEQ ID NO: 21), DQKDSGD (SEQ ID NO: 22), CPYVPNANQADHDKDGKGDA (SEQ ID NO: 23), and a portion, at least 3 amino acyl residues in length of such an amino acid sequence.

161. The method of Claim 26 wherein in Step 2 the epitope comprises an amino acid sequence selected from the group consisting of a fragment of claim 8, TERDDD (SEQ ID NO: 24), DFGTFFINTERDDD (SEQ ID NO: 25), ERKDHS (SEQ ID NO: 26), TRGTLLALERKDHS (SEQ ID NO: 27), (C)TRGTLLALERKDHS (SEQ ID NO: 28), DDKFQD (SEQ ID NO: 29), ANLIPPVPDDKFQD (SEQ ID NO: 30), (C)ANLIPPVPDDKFQD (SEQ ID NO: 31), DCEKME (SEQ ID NO: 32), EDRAQLYIDCEKMEN (SEQ ID NO: 33), CGTNRIPESGGDNSVFD (SEQ ID NO: 34), NRIPESGGDNSVFD (SEQ ID NO: 35), GWKDFTAYRWRLSHRPKTG (SEQ ID NO: 36), CGWKDFTAYRWRLSHRPKTG (SEQ ID NO: 37), DDDDNDKIPDDRDNC (SEQ ID NO: 14), DDDDNDKIPDDRDNC[NH₂] (SEQ ID NO: 15), DDDDNDK (SEQ ID NO: 16), NLPNSGQEDYDKDG (SEQ ID NO: 17), CNLPNSGQEDYDKDG (SEQ ID NO: 18), EDYDKD (SEQ ID NO: 19), CPYNHNPQADTDNNGEGD (SEQ ID NO: 20), YAGNGIICGEDTDLD (SEQ ID NO: 7), EDTDLD (SEQ ID NO: 6), CNSPSPQMNGKPCEGEAR (SEQ ID NO: 8), PQMNGKPCEGEARE (SEQ ID NO: 5), EGEARE (SEQ ID NO: 4), PQMNGKPCEGEAR (SEQ ID NO: 11), CEGEAR (SEQ ID NO: 12), CRLVPNPQKDSGD (SEQ ID NO: 21), DQKDSGD (SEQ ID NO: 22), CPYVPNANQADHDKDGKGDA (SEQ ID NO: 23), and a portion, at least 3 amino acyl

residues in length of such an amino acid sequence, provided that said peptide with a designated SEQ ID NO. does not comprise an epitope used in step 1.

162. A method of Claim 137 wherein said protein comprises a single chain antibody.

163. A method of Claim 60 wherein the binding agent is an antibody induced by either thrombospondin, a modified form of thrombospondin, a fragment of thrombospondin, a fragment of a modified form of thrombospondin, or a modified fragment of thrombospondin.

164. A method of Claim 163 wherein a modified form of thrombospondin was generated by glycosylation, deglycosylation, β -hydroxylation, denaturation, renaturation, calcium depletion, calcium supplementation, alkylation, reduction, conjugation, and/or addition of groups or moieties to aid conjugation, stability, and/or immunogenicity of thrombospondin; and wherein a modified fragment of thrombospondin was generated by glycosylation, deglycosylation, β -hydroxylation, alkylation, reduction, conjugation, and/or addition of groups or moieties to aid conjugation, stability, and/or immunogenicity of said fragment.